

Synthesis and Optimization of Nisin-Silver Nanoparticles at Different Conditions

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ABSTRACT

Silver nitrate and commercial sigma Nisin were used to synthesize Nisin-Silver nanoparticles; different concentrations of Nisin at different pH conditions were used to optimize the characterization. 10 µg/ml at pH 6.0 had the greatest observation and the deferent conditions characterization of Nisin-Silver nanoparticles agreed with that different methods were used to prepare the Nisin-Silver nanoparticles and the results showed that the best method to prepare the nanoparticles (Nisin-Silver) was sunlight method.

Keyword: Silver, Nisin, loaded nanoparticles.

تصنيع وتوليف مركب الناييسين – فضة النانوية تحت الظروف المختلفة

الخلاصة:

استخدمت نترات الفضة جنباً إلى جنب مع بروتين الناييسين لتصنيع جسيمات الناييسين – فضة النانوية، عدة تراكيز من الناييسين استخدمت تحت ظروف حامضية متعددة لتحسين خصائص الجسيمات المنتجة باستخدام طرق مختلفة لإنتاجها. وأوضحت طرق التصنيع التي استخدمت في الدراسة أن تصنيع جسيمات الناييسين – فضة النانوية تحت أشعة الشمس أعطى أفضل النتائج مقارنة ببقية الطرق وتركيز (10 ميكروغرام \ مل) من الناييسين في درجة حموضة (6).

INTRODUCTION

The field of nanotechnology has recently witnessed spectacular advances in the methodology of nanomaterials fabrication & utilization of their exotic physiochemical and optoelectronic properties ⁽¹⁾. With the development of new protocols based on chemical or physical methods, very much concern for environmental contamination are also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous by-products. Thus, there is a need for “green chemistry” that includes a clean, nontoxic, and environmentally-friendly method of nanoparticles synthesis ⁽²⁾. As an alternative to conventional methods,

biological methods are considered safe and ecologically sound for the nanomaterials fabrication⁽³⁾. One of the prime challenges for researchers in biosynthetic method is the systematic size and shape control of inorganic metal nanoparticles. Control over the shape and size of metallic nanoparticles enables tuning of their optical⁽⁴⁾, electronic⁽⁵⁾, magnetic⁽⁶⁾ and catalytic⁽⁷⁾ properties. There are some parameters like pH, temperature and concentration of reactant that can be varied to tune the driving force over a large range of values. The driving forces can be quantified by calculating the free energy available for the reaction and thus regimes where different mechanisms will be operative can be identified. The existing physical methods for metal nanoparticles synthesis such as gas condensation and irradiation ultraviolet or γ radiation are usually associated with low production rate and high expense⁽⁸⁾. The physical and chemical methods for synthesis of metal nanoparticles include chemical reduction of metal ions in aqueous solutions with or without stabilizing agents, thermal decomposition in organic solvents, chemical and photoreduction in inverse micelles and radiation chemical reaction. However, in view of the rapid progress of application of nanomaterials in different fields, there is a growing need to develop clean, nontoxic, simple & environmental friendly procedures for the synthesis and assembly of nanoparticles. Towards this objective, the present study is focused on the extracellular synthesis of metal nanoparticles using such bio-based protocols. Commercial nisin have been used for the extracellular synthesis of nanoparticles.

Synthetic Methods

Chemical Materials

Silver nitrate, Nisin (1×10^6 IU/g) in the form of Nisaplin were obtained from Sigma-Aldrich-UK, double distilled water.

Preparation of aqueous

Silver nitrate:

1 mM AgNO_3 solution was prepared and stored in an amber color bottle.

Preparation of Nisin sample:

The lyophilized Nisin powder was taken in different concentrations and dissolved in double distilled water & used for the optimization studies.

Synthesis and optimization of Nisin-Silver nanoparticles compounds:

1 mM AgNO_3 solution was prepared and different concentrations of Nisin 10 mg, 50 mg, 100 mg was taken and dissolved in 10 ml of double distilled water respectively. 1 ml, 3 ml and 5 ml of protein solutions were taken from 10 mg/10 ml concentration in separate conical flask and 10 ml of 1 mM AgNO_3 solution was added with constant stirring and exposed to different conditions including pH 4, 6, 8 and 10.

Synthesis of Silver nanoparticles under Room temperature

The samples mentioned above are incubated under Room temperature for 2-3 days and observed the color change. The color change of the solution was checked at every 24 hrs, the solution was monitored and measured by using UV-Vis spectra then the conical flask

was incubated at room temperature for another 48 hours until the completion of the reaction. The same was performed for 50 mg/10 ml and 100 mg/10 ml concentrations.

Synthesis of Nisin-Silver nanoparticles under Sunlight irradiation

The samples are exposed to sunlight radiation and observed for the color change. The color change of the solution was checked periodically, the solution was monitored and measured by using UV-Vis spectra then the conical flask was incubated at room temperature for 48 hours until the completion of the reaction. The same was performed for 50 mg/10 ml and 100 mg/10 ml concentrations.

Synthesis of Silver nanoparticles under Direct Boiling

The samples are undergone direct boiling and observed for the color change. The color change of the solution was checked periodically the solution was monitored and measured by using UV-Vis spectra then the conical flask was incubated at room temperature for another 48 hours until the completion of the reaction. The same was performed for 50 mg/10 ml and 100 mg /10 ml concentrations.

Synthesis of Silver nanoparticles under UV irradiation

The samples are exposed under UV radiation and observed for the color change. The color change of the solution was checked periodically, the solution was monitored and measured by using UV-Vis spectra then the conical flask was incubated at room temperature for another 48 hours until the completion of the reaction. The same was performed for 50 mg/10 ml and 100 mg/ 10 ml concentrations.

Synthesis of Silver nanoparticles under Microwave irradiation

The whole mixture was put in a domestic microwave oven (National Model N N-GD 576M). The sample was subjected to several short bursts of microwave irradiation at a frequency of 2.45 GHz, at a power output of about 100W in a cyclic mode (on 15 s, off 15 s) to prevent overheating as well as aggregation of metals. The irradiation process was conducted for a minimum of 5 up to a maximum of 15 cycles and observed for the color change. The color change of the solution was checked periodically, the solution was monitored by measuring using UV-Vis spectra then the conical flask was incubated at room temperature for another 48 hours until the completion of the reaction. The same was performed for 50 mg/10ml and 100 mg/10 ml concentrations.

Physio-chemical characterization of Nisin-Silver nanoparticles

Surface plasmon resonance of the samples was studied with a UV-Vis Spectrophotometer (Cary 300 Conc spectrophotometer) at a resolution of 1 nm from 250 nm to 800 nm. Characterization of the morphology of the nanoparticles was carried out using a QUANTA 200 FX MARK II microscope operating at 20.00 KV (for high resolution SEM imaging) with EDAX. The size of nanoparticles was preformed with Atomic Force Field Microscopy (AFM), the sample was prepared by placing a drop of colloidal solution of nanometal on a carbon-coated copper grid and was completely dry in the vacuum desiccators. The image of the sample was obtained using Atomic Force electron Microscope equipped with an XRD. The infrared (IR) spectra of the crude

extracts were obtained (as KBr discs) between 400-4000 cm⁻¹ on a Perkin Elmer 2000 FT-IR Spectrophotometer. The important IR bands, such as $\nu(\text{C-N})$, $\nu(\text{O-H})$, $\nu(\text{C-H})$, $\nu(\text{C=C})$, $\nu(\text{N-H})$, $\nu(\text{C-O})$ and (C-H) the symmetric and asymmetric stretching, and the stretching frequencies were studied to determine the presence of functional groups in the ethyl acetate crude extracts.

RESULT

Study of Nisin-Silver Nanoparticles compounds characterization

Nisin-Silver Nanoparticles Synthesis

Five different concentrations of Nisin (1, 2.5, 5, 7.5 and 10 mg/1 ml) were used in the preparation of silver nanoparticles irradiated by sunlight. The results revealed that 10 mg/ml of 1 mM AgNO₃ was very effective and induced enhanced synthesis of the nanoparticles (Fig.1.a and b). Hence, it was chosen for bulk production.

Effect of pH

The pH of the reaction solution strongly affects the driving force of the reaction. The pH was found to increase the driving force and hence favors the reaction. In all pH (4, 6, 8 and 10) optimizing methods conducted, pH 6 provides the best condition to complete reaction and that is shown in Fig.2. From the results suggested by the figure, it could be noted that formation of silver nanoparticles was much lower in acidic condition compared with (pH 8 and 10). This may be due to the role of acidity in effect on atomic charge, ionic legends and driving force.

Spectral Properties of the Nisin-Silver Nanoparticles

Figs.3. Shows a strong surface plasmon centered around 450 NM. This indicates the formation of nisin-silver nanoparticles. It is not possible to detect nisin at 280 nm since it does not contain any aromatic amino acids, the 215 nm was chosen.

X-Ray Diffraction (XRD) Analysis

The reduction of Ag⁺ of the ions elemental silver by nisin was studied by the XRD analysis (Fig.4) shows the X-ray diffraction pattern of the sample. The spectrum shows eight major diffraction intensity peaks at $2\theta = 27.810^\circ$, 32.165° , 38.439° , 41.334° , 45.991° , 54.470° , 57.085° and 66.967° . The peaks were identified to originate from (2 1 0), (1 2 2), (1 2 3), (3 1 1), (2 3 1), (0 0 6), (4 1 2) and (3 3 4) planes of the sample, respectively (JCPDS no: 84-0713). The information on the particle size was obtained from the full width at half maximum (FWHM) of the diffracted beam using Sherrer Formula⁽⁹⁾:

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

D: is the mean size of the ordered (crystalline) domains, which may be smaller or equal to the grain size.

0.9: is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystalline.

λ : is the X-ray wavelength.

B: is the line broadening at half the maximum intensity (FWHM), after subtracting the instrumental line broadening, in radians. This quantity is also sometimes denoted as $\Delta(2\theta)$.

θ : is the Bragg angle.

The sample displayed an average crystalline size of 32.9935 nm.

High Resolution Scanning Electron microscopy (HRSEM)

Fig.5. Shows the HRSEM image of the Silver - Nisin sample. The image shows that all the particles are spherical in shape on a nanometer scale. There is a bright image particle surrounding the dark image particle that may correspond to the coating of silver on Nisin. The image also indicates that the silver ions interact with the Nisin protein.

Atomic Force Electron Microscopy (AFM)

The AFM micrograph obtained for the Nisin-silver nanoparticles (Fig.6) indicates the surface roughness changes and the surface roughness change [root mean square (Rp)] values were identified. For the sample the roughness value was 56 nm and the section analysis of the sample's grain size value was 58 nm.

Fourier Transform Infrared spectroscopy

The FTIR spectrum of bacterial Nisin is shown in (Fig. 7). The intense broad peak at 3450 cm^{-1} was due to the N-H stretching vibration of amide groups. The broadening was due to hydrogen bonding. The C-H stretching vibration of alkyl groups occurred at 2964 cm^{-1} . The C=O vibration of amide groups yielded their peak at 1652 cm^{-1} . The N-H bending vibration was seen at 1540 cm^{-1} . The CH₂ bending vibration occurred just below 1500 cm^{-1} . The peaks between 1300 and 1000 cm^{-1} were due to C-N and C-O vibrations. The broad peak at 662 cm^{-1} was due to S-S (disulfide) vibration of Nisin protein. The FTIR spectrum of the samples was recorded by Perkin-Elmer Fourier transform infrared spectroscopy. The presence of any functional group as revealed by IR spectral is shown in (Fig.7). The FTIR Spectral analyses of Sample shows certain common absorption bands in the range of 3904 - 3428 cm^{-1} is a characteristic of hydroxyl ν (O-H) and ν (N-H) vibrational frequency which are interchangeable. A common vibrational peak at 2925 cm^{-1} is characteristic of a ν (C-H) symmetrical vibration of saturated hydrocarbon. The vibrational frequency ν (C=O) was observed in the spectra at 1617 cm^{-1} which is corresponding to CO stretching. The 1379 cm^{-1} peak revealed the C-N and C-O vibrations Band stretching.

DISCUSSION

In this study 1 mM AgNO₃ solution was added to the Nisin solution to make Nisin - Silver nanoparticles in the biological methods under sunlight. Color change of the solution from colorless to dark brown indicated the silver generation of nanoparticles from the protein. Several factors, including Nisin concentration and pH might influence the production and the dimensions of Nisin -Silver nanoparticles. The intensity in

absorption peaks increases when the concentration of Nisin is incurred clearly indicates a complete reduction of silver nanoparticles when they react with higher concentration of Nisin. The variation in the size of nanoparticles depends on the pH of the solution. Furthermore, particles obtained at pH (6.0) were smaller. But polydispersity decreased by lowering the pH (4.0)⁽¹⁶⁾. Silver nanoparticles are stable at neutral pH but not in acidic pH which can be attributed to the stability of the capping protein. The broadening of absorbance with a little red shift of the absorption maxima is observed for silver nanoparticles solution indicating the aggregation of the particles. Thus, it can be concluded from the above observation that Nisin solution for capping silver nanoparticles is stable at the neutral pH, but not in the acidic or basic pH which can be attributed to the stability of the capping proteins.

UV-Visible spectrum was measured for the reaction mixture of the Nisin-silver nanoparticles using 1, 2.5, 5, 7.5 and 10 mg/ml of Nisin solution was exposed to 1 mM AgNO₃. It can be observed that in the silver ions exposed to 10 mg/ml to 2.5 mg/ml of Nisin, the observance of surface plasmon resonance of the reaction mixture showed an apparent broadening and red shift at 450 nm indicating the aggregation of silver nanoparticles but where when, the concentration was raised to 5 to 10 mg/ml, no broadening or red shift of observance occurred. That indicated that a small amount of Nisin (1 – 2.5 mg/ml) reduced the Ag³⁺ ions to Ag⁰ but the aggregates indicated the non-availability of the requisite quantity of the capping proteins.

The color of the reaction mixture changed from colorless to dark brown. Which indicates the formation of Nisin -loaded Silver nanoparticles. It is well-known that silver nanoparticles exhibit dark brown color in water due to the excitation of the surface plasmon vibration of the silver nanoparticles⁽¹⁰⁾. UV-Visible spectroscopy indicated the strong surface plasmon resonance centered on a. 450 nm that is the characteristic of the colloidal silver. The spectra clearly show the increase in intensity of silver solution with time, indicating the formation of increased number of silver nanoparticles in the solution. The excitation spectra of the Nisin-loaded silver nanoparticle sample were obtained by the UV-Visible spectroscopy and this technique proves to be useful for the analysis of the nanoparticles⁽¹¹⁾. The UV- Visible spectroscopy of the Nisin-Silver nanoparticles was consistent with the X-ray analysis, which could be indexed to P6₃mc (61) face group (Face-centered) orthorhombic structure, with cell parameter $a = 7.1999 \pm 0.0430 \text{ \AA}$, $b = 6.8043 \pm 0.00837 \text{ \AA}$, $c = 10.2880 \pm 0.4600$ and $v = 504.011$ The crystalline size is calculated by the Debye-Scherrer formula. The sample gives the average crystalline size was found to be 24.1836 nm.

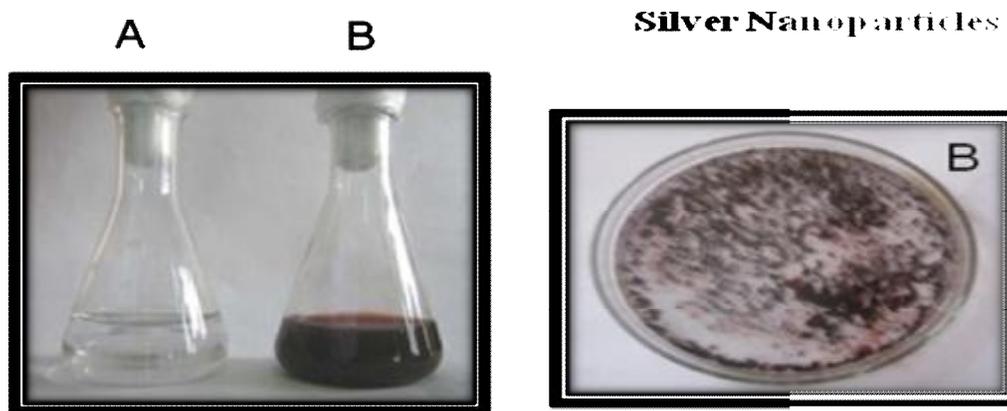
FTIR Spectroscopic studies were carried out to investigate a possible bioreducing agents presence in the extract. The spectra of the extracts were recorded before and after adding silver nitrate (a) and chloroauric acid (b) solution. Both the interferogram exhibited a broad bond at 3394 cm^{-1} that is assigned to the N-H group from the peptide linkage present in the extract. Formation of the C=C bonds is energetically favored over S-C bonds as the latter could impose severe geometrical constraints on the molecule more being specific in thiol group and less in the acidic as compared to the alcohols and that makes elimination of the hydrogen that is attached to the sulfur group. There is a decrease in the concentration of the amide linkage in the aqueous solution after the generation of the silver nanoparticles.

The spectrum showed the presence of bands hydroxyl ν (O-H) ν (N-H) vibrational frequency, C=O and S-C bonds vibration present in the amide linkages of the proteins, respectively. The position of this band is close to that reported in the literature for native proteins⁽¹²⁾. Thus, the FTIR measurement indicated that the secondary structure of proteins was not affected because of its interaction with Ag⁺ and Au⁺ ions or the nanoparticles. The finding in this study provides a basis for understanding the biological activities by identifying protein residue as an important and a common reducing source in the system. Jianping et al. Concluded⁽¹³⁾ that the hydroxyl group of tyrosine was active in the reduction of metal ion. From the results, it was confirmed that Nisin hydroxyl group was involved in the silver and gold ion reduction and this is supported by evidence in the biological system including bacteria^(3 and 14).

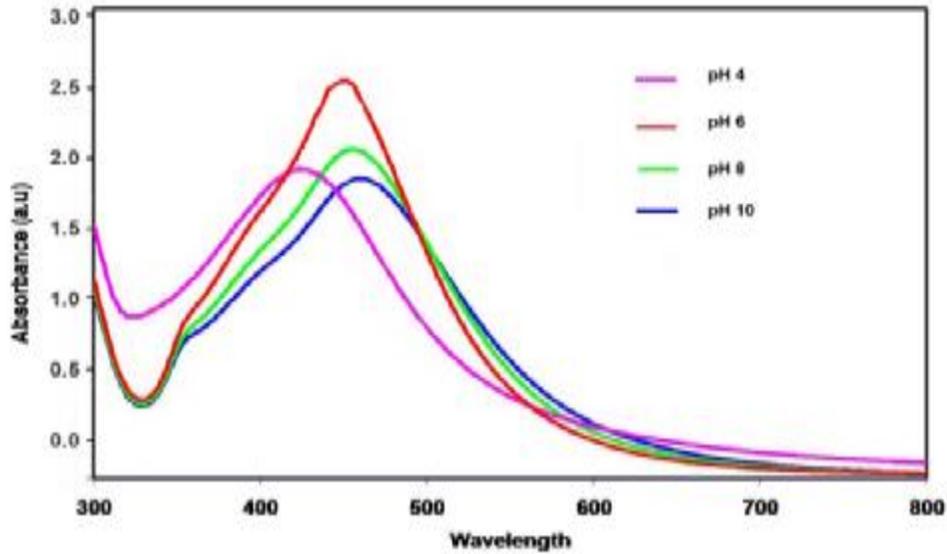
A pre-requisite for most of these studies and thus an extremely important feature of these particles is their superb stability at ambient conditions resulting from the very effective surface passivation by the thiol capping agent. Most research to date has focused on non-polar, alkane thiol-capped particles of 1-4 nm diameter, which are insoluble in water. These are usually referred to as Monolayer Protected Clusters (MPCs). Their unique properties have been widely established only for materials of this composition and size range. Considering the broad range of technological applications that are envisaged for MPCs, those related to bioanalytical and biomedical problems appear to be the most promising at present⁽¹⁵⁾.

CONCLUSION

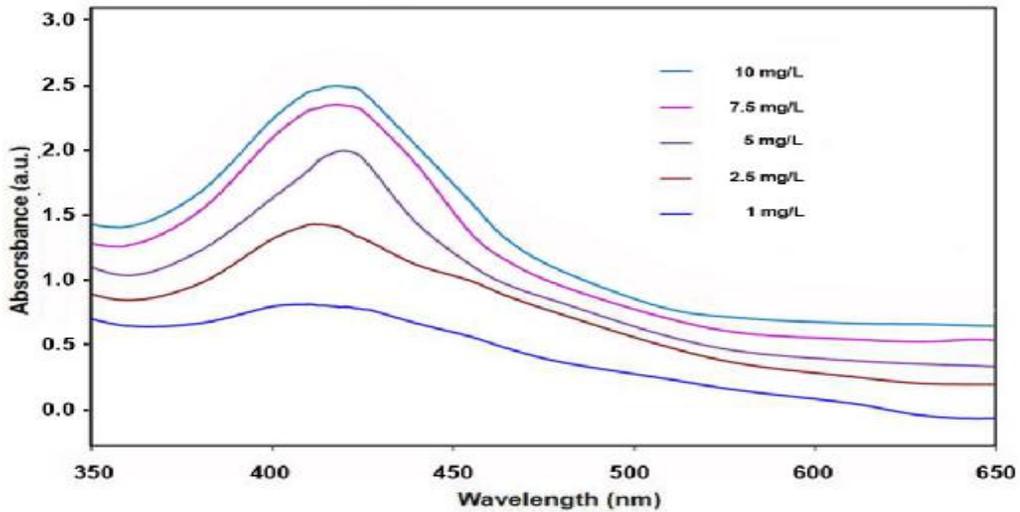
In this paper, it has been shown for the first time use of Nisin in synthesis of Silver nanoparticles under different conditions. The synthesis and stability of nanoparticles solution under sun irradiation method is due to secretion of reducing linkage in a protein. The stability of the nanoparticle is found to be pH dependent. At higher pH the nanoparticles solution remains stable while it aggregates at lower pH as the protein gets denatured. The advantages are the methods used of synthesis the metal nanoparticles.



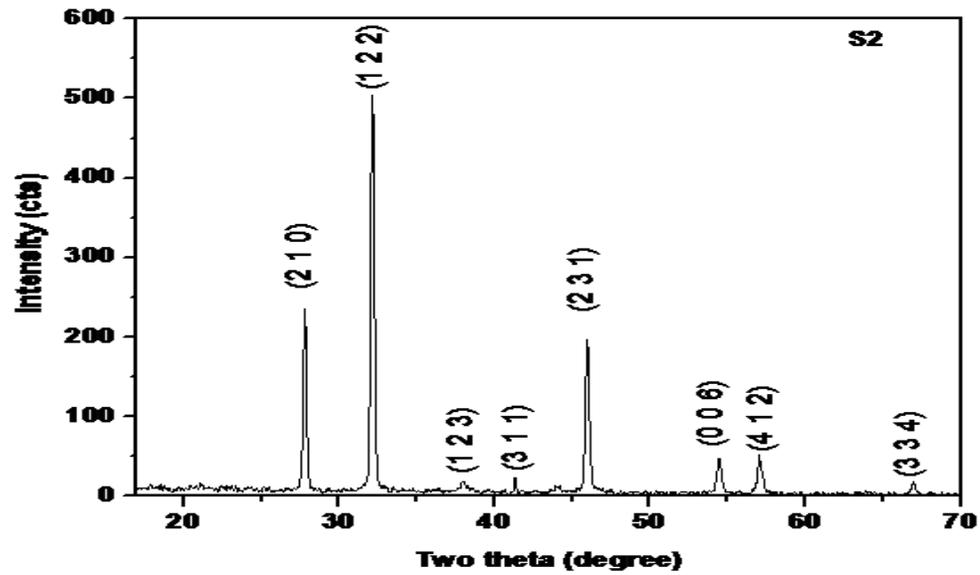
Figure(1). Synthesis of nisin-silver nanoparticles (a):before reaction (b):after reaction



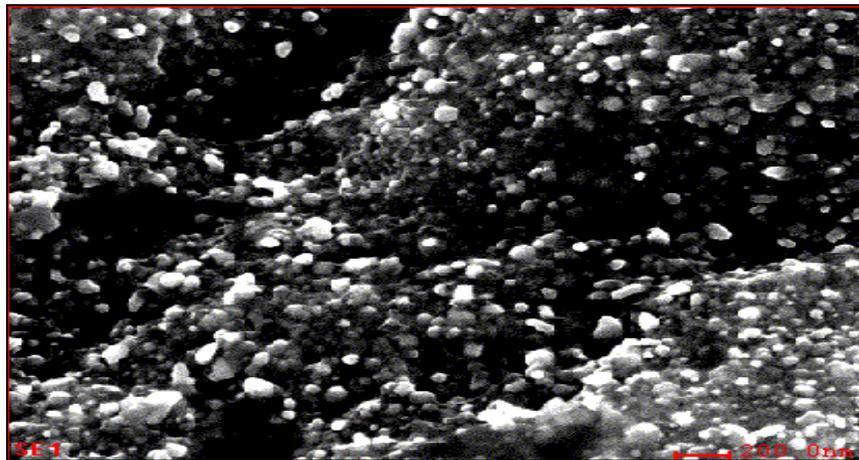
Figure(2).Absorption spectra of commercial nisin based silver nanoparticles at different pH in sunlight method.



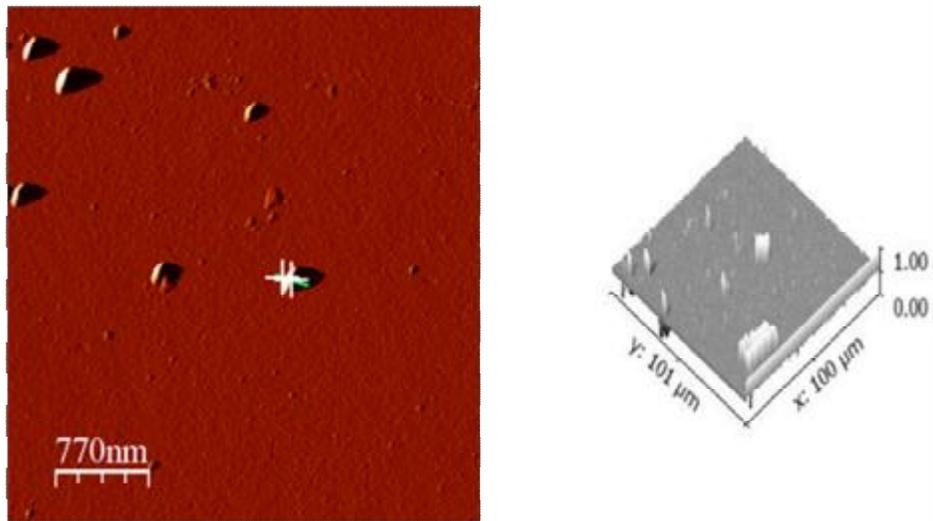
Figure(3).Absorption spectra of commercial nisin based silver nanoparticles at different nisin concentration in sunlight method.



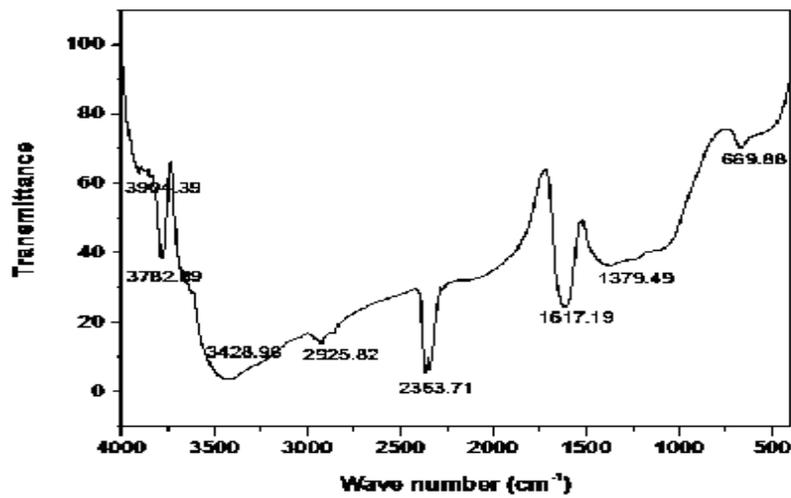
Figure(4). XRD of nisin-silver nanoparticles.



Figure(5). HRSEM of nisin-silver nanoparticles.



Figure(6). AFM for nisin-silver nanoparticles.



Figure(7). FTIR nisin- silver nanoparticles.

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